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TITLE: Improving Outcome in Malignant Pleural Mesothelioma (MPM) Using Pulsed-Protracted External Beam Radiation (PERT) and Intrapleural Delivery of Stem Cells

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14. ABSTRACT

Malignant Pleural Mesothelioma (MPM) survival remains poor despite multidisciplinary treatment involving aggressive surgery, chemotherapy and adjuvant radiotherapy (RT). The large RT treatment volume, and concerns about the proximity of radiosensitive normal structures, restricts the tumoricidal dose of radiotherapy that can be delivered. These concerns limit the effectiveness of adjuvant RT. To overcome this limitation, an entirely novel radiation treatment schedule in combination with post-RT delivery of bone marrow-derived stem cells was examined to improve tumor control and facilitate normal tissue proliferation. A rat model of MPM was used. The RT regime consisted of 10 pulses of low-dose RT (0.2 Gy) using a 3 minute inter-pulse interval (PERT) to a daily dose of 2 Gy. The inclusion of post-RT stem cell therapy is to repopulate normal tissues in the RT field. RT tumor response was assessed by microPET/CT (Positron emission tomography/computed tomography) imaging. In vitro cell survival data was used to demonstrate PERT was not inferior to standard RT (2 Gy single continuous treatments). In vivo, The surgical procedure has been established and tumor model has been established and tumor volume determined by in situ with F18-FDG. Unexpected technological problems with respect to the microPET scanner have slowed the imaging aspect of the project. However, to date, we have demonstrated that RT is effective at reducing MPM tumor growth in vivo; and this is associated with recruitment of hematological stem cells. Studies are currently on-going to determine if PERT is superior to standard RT in MPM.

15. SUBJECT TERMS

Malignant Pleural Mesothelioma, Low dose pulsed radiotherapy, stem cells, animal imaging.

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ABSTRACT

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INTRODUCTION

Malignant Pleural Mesothelioma (MPM) survival remains poor despite multidisciplinary treatment involving aggressive surgery, chemotherapy and adjuvant radiotherapy (RT). The large RT treatment volume, and concerns about the proximity of radiosensitive normal structures, restricts the tumoricidal dose of radiotherapy that can be delivered. These concerns limit the effectiveness of adjuvant RT. We propose to deliver the radiotherapy using an entirely novel treatment schedule and combine this with post-RT local-delivery of bone marrow-derived stem cells to facilitate normal tissue proliferation. The concept is to deliver 10 pulses of low-dose RT (0.2 Gy) using a 3 minute inter-pulse interval (PERT) to introduce the RT-induced damage at a

level that evades ATM dose-dependent DNA damage detection and repair mechanisms. Post-RT stem cell therapy is to repopulate normal tissues in the RT field.

BODY

The surgical procedure has been established. Animals are anesthetized, surgically-implanted with tumor cells and recovered. These tumors were imaged *in situ* with F18-FDG (Fig. 1).

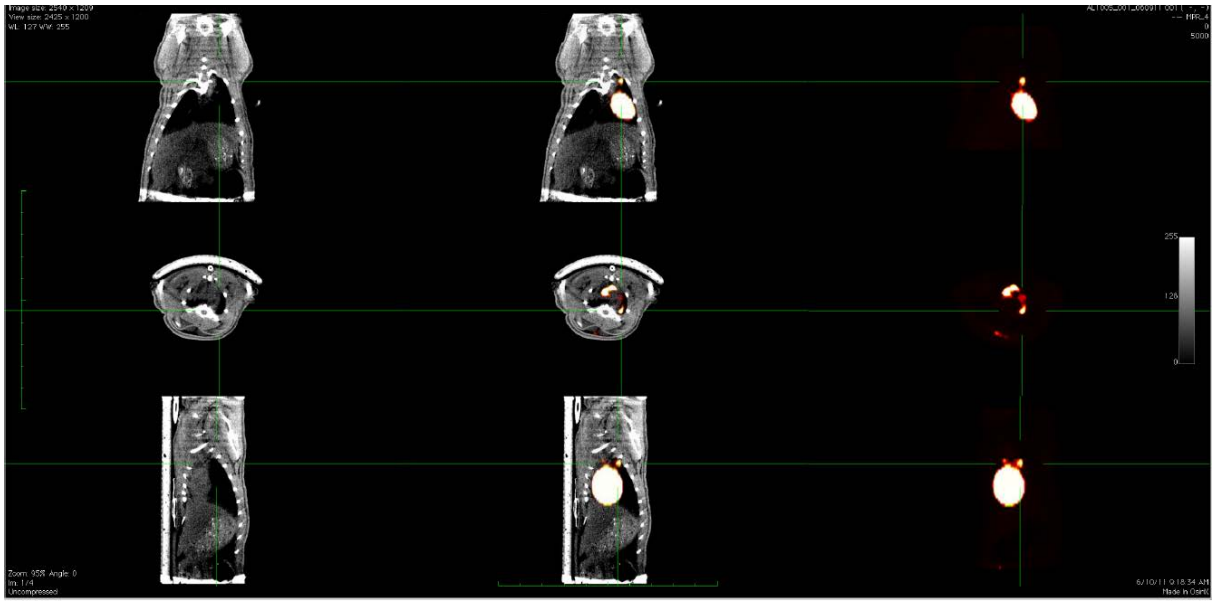

Fig. 1: F18-FDG uptake (bright image) in the lung after tumor implant

As the project was initiated, a number of unexpected technology problems were encountered that slowed the imaging progress of the project. Two state-of-the-art pieces of equipment essential for the project, a small animal radiation research platform (CT image guided X-irradiation device) and microPET/SPECT/CT imaging machine, failed. Repairs took a number of months and restricted the extent of the imaging that could be achieved. In addition, Dr. John Chunta the microPET scientist working on the project was recruited by another institute. The technical and staff problem have now been resolved and the scanning is progressing.

The proposal stated that bone marrow stem cells would be harvested from donors using flow-cytometry ‘side population’ characteristics and then injected into recipient animals that had been irradiated. The aim was then to follow the recruitment of the stem cells to sites of radiation damage. We pursued the stem cell transplant aspect of the project with success. To test the methodology, stem cells were isolated from a green fluorescent protein transgenic animal and

injected into normal recipients. The GFP-labeling of the stem cells allowed their recruitment to be quickly and easily seen in lung tissues using fluorescent microcopy. We were able to see transplanted stem cells in the irradiated lung up to 8 weeks after radiation treatment. These experiments have defined the stem cell sorting and transplantation procedures and methods needed for the project (Fig. 2).Immunohistopathology for F4/80, CD68, CD34, and VEGF has been developed and an automated scoring system developed using NIH imageJ.

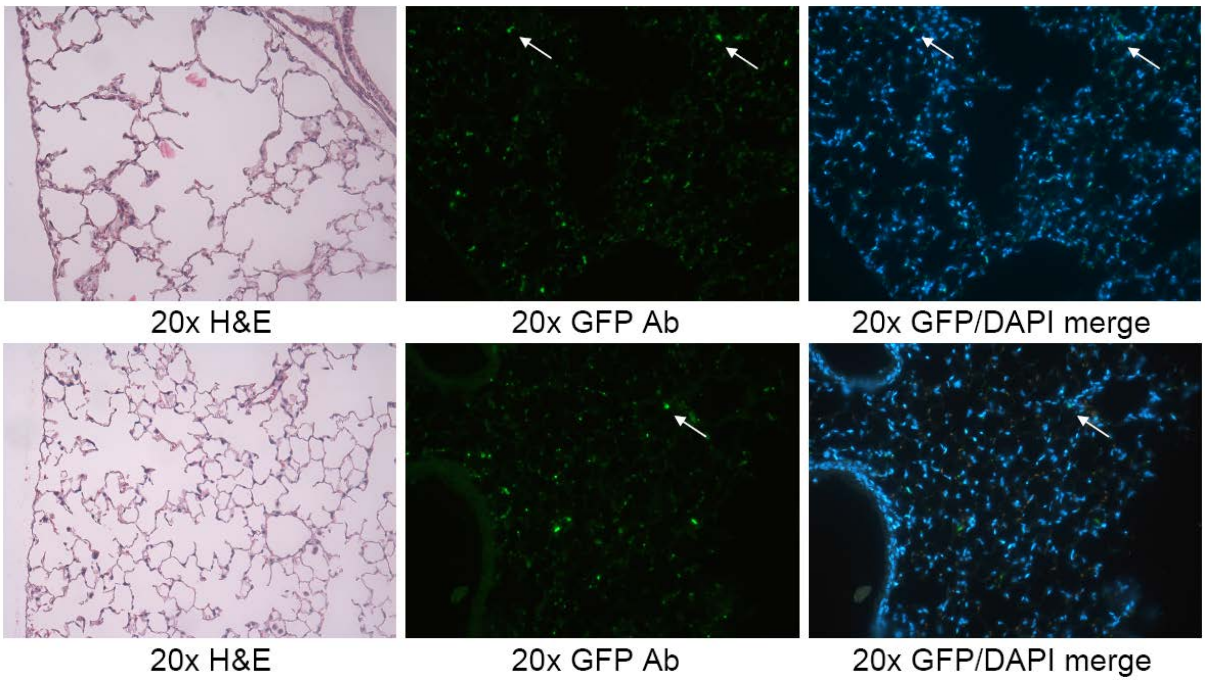

Fig. 2: Incorporation of GFP-stem cells (green) in to the lung of irradiated animals. Blue indicates DAPI labeled cells.

We have evaluated the radiation procedures of administering a low-dose pulsed treatment regime using the micro-irradiator. Animals are anesthetized during irradiation procedure (10 doses of 0.2 Gy given daily for 5 consecutive days) with 2-3% isoflurane; this procedure has been defined and is well-tolerated. This procedure is now established.

KEY RESEARCH ACCOMPLISHMENTS

- Measure survival response of MPM cells in vitro
- Demonstrated PERT is not inferior to standard RT in vitro
- Established animal model

- Demonstrated RT reduces MPM growth in vivo
- Demonstrated recruitment of stem cells to sites of radiation damage in vivo

REPORTABLE OUTCOMES

At present, the work is on-going and a no-cost extension has been requested. Therefore the wok has not been published or presented.

CONCLUSIONS

The experiments are still on-going. We have demonstrated that PERT is not inferior to standard RT (the current standard of care for MPM). The preliminary in vivo data are supportive of this conclusion. Stem cell recruitment occurs in normal tissues, although the significance of this recruitment has yet to be determined with respect to tissue function.

FUTURE EXPERIMENTS

$1x10^6$ Il-45 cells will be injected into the pleural of Fischer 344 rats, and allowed time to growth. Once tumor has been confirmed by microPET imaging, animals will be irradiated using two RT schedule (single 2 Gy fraction per day) or PERT (10 x 0.2 Gy to same daily dose) with or without stem cell delivery (n=10 per arm) to a total dose of 30 Gy. Treatment response will be evaluated and harvested tissues examined for incorporated stem cells using the techniques described above.

TIME LINE

We expected the remainder of the experiments to be complete within the next few months as all the assays and procedures have been established.

REFERENCES AND APPENDICES

None. Studies are on-going and data is still being compiled for publication.

WITH RESPECT TO STATEMENT OF WORK

Specific Aim #1 – Establish and treat Mesothelioma model (Months 1-7)
Overview. The aim is to develop Mesothelioma model and treat with pulsed radiotherapy.

Subtask1: Establish surgical technique and tumor implantation (Months 1-2)

a. Purchase and acclimatize 12-week old rats.
b. Establish surgery procedure for intrapulmonary implanting of Mesothelioma cells.
c. Ensure successful infection-free surgery without adverse pulmonary breathing rates or events.

The work from Subtask1 is complete.

Subtask2: Compare Radiation schedules (Months 2-7)

a. Measure breathing rate and lung function in tumor-bearing animals
b. Obtain weekly blood samples
c. Treat with conventional fractionated radiotherapy and pulse schedules
d. MicroPET scan animals to assess treatment outcomes
e. Harvest lungs and other tissue for histopathology
f. Analyze data set to determine effectiveness of two RT schedules.

The work from Subtask2 is partially complete. Tasks a, b, d, e, have been completed. Tasks c and f are not complete but these are underway. Data analysis will begin when all animals have been irradiated and sacrificed.

Subtask3: Compare Radiation schedules (Months 2-10)

g. Measure breathing rate and lung function in tumor bearing animals
h. Obtain weekly blood samples
i. Treat with conventional fractionated radiotherapy and pulse schedules ***plus stem cell therapy***
j. MicroPET scan animals to assess treatment outcomes
k. Harvest lungs and other tissue for histopathology
l. Analyze data set to determine effectiveness of two RT schedules.

The work from Subtask3 is partially complete. Tasks g, h, k, have been completed. Task i is not complete but stem cells have been successfully harvested and given to recipient animals. Data analysis (subtask l) will begin when all animals have been irradiated and sacrificed.

Specific Aim #2 – Analysis microPET images and compare with histology (Months 10-12)
Overview. Compare outcomes of difference RT schedules in the presence and absence of stem cells.

Specific Aim #2 examines excised tumors using histopathology and compares with microPET analysis (Months 10-12)

Subtask1: Surgically excise tumor (regrowth) and normal tissues from treatment animals.

a. Surgically extract intrapulmonary tumors
b. Block and section tissue for histological examination
c. Cut sections and stain with H&E and specific immunochemistry

Subtask2: Compare histology with non-invasive tumor imaging (Months 3-12)

a. Mathematically compare **histology** with functional SUV imaging data
b. Analyze entire data set to determine if PERT is more effective than conventional RT for tumor regrowth and the outcome of stem cell therapy, as confirmed by histology and microPET/CT imaging.

The work from Aim 2 is partially complete. Tasks a, b, c, have been completed for those animals used to develop the assay. Sub-tasks a and b are not complete Final data analysis and statistical comparisons will begin when all animals have been irradiated and sacrificed.